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Responses of *Nicotiana tabacum* to CO_2 enrichment at low-photon flux density

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Effects of CO_2 enrichment on photosynthesis and on dry matter allocation were examined in two tobacco (*Nicotiana tabacum* L.) genotypes, Samsun and W38. Plants were grown from seed in controlled environment chambers at a photosynthetic photon flux density of 450 μmol m^{-2} s^{-1}. Averaged over the 9 day study, net photosynthesis rates were 14.2 ± 0.5 and 13.0 ± 0.4 μmol m^{-2} s^{-1} in elevated (70 Pa) and in ambient (35 Pa) CO_2 air, respectively, when measured at the irradiance and CO_2 partial pressure employed for plant growth. However, photosynthesis rates of plants grown in elevated CO_2 were 50% less than those of the ambient controls on the last day of treatment, when measured at 70 Pa CO_2 air and an irradiance of 900 μmol m^{-2} s^{-1}. Total plant dry weight and specific leaf weight were greater ($P < 0.05$) in enriched-CO_2-grown than in ambient-CO_2-grown plants. Leaf starch, measured during the first hour of the photoperiod, increased over 7 days of treatment in elevated-CO_2-grown but not in ambient-CO_2-grown plants. Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activities of tobacco plants grown at 35 and 70 Pa CO_2 air were 58.5 ± 4.5 and 48.5 ± 3.7 μmol m^{-2} s^{-1}, respectively, between days 0 and 9 of the study. Rubisco activation state, Rubisco protein concentration, soluble protein and total chlorophyll were unaffected by CO_2 enrichment. The above findings demonstrated that photosynthesis was down regulated in tobacco plants after 7 to 9 days of CO_2 enrichment at low photosynthetic photon flux density, but less than at moderate irradiances.

Key words – Carbohydrate metabolism, CO_2 enrichment, *Nicotiana tabacum*, photosynthate partitioning, photosynthetic acclimation, Rubisco.

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Introduction

Atmospheric CO_2 concentrations have risen about 25% in the last 100 years and a doubling to 70 Pa CO_2 is expected during the next century (Watson et al. 1990). Rising CO_2 levels are a potentially important factor in plant growth, water use efficiency and agricultural or ecosystem productivity (Bazzaz and Fajer 1992). However, increased photosynthetic rates and the growth enhancement of plants often diminish after days to weeks of CO_2 enrichment in controlled environment studies (Kramer 1981, Sage et al. 1989). Although less consistently, this phenomenon has been observed in field experiments (Rowland-Bamford et al. 1990).

The above observations have led to widespread interest in identifying biochemical factors that are involved in the down regulation of photosynthesis during growth in elevated CO_2. The majority of studies (see reviews by Bowes 1991, Stitt 1991) on this subject have focused on changes in the activity of the principal CO_2 fixing enzyme in the leaf, Rubisco (EC 4.1.1.39). Changes to Rubisco during growth in CO_2-enriched air are diminished leaf enzyme concentrations, decreased carbamylation state and decreased specific activity of the enzyme (Sage et al. 1989, Yelle et al. 1989b, Rowland-Bamford et al. 1990, Sicher et al. 1994).

Several investigators have reported that negative changes in growth and in photosynthetic rates occur in CO_2 enriched atmospheres at irradiances less than 500 μmol m^{-2} s^{-1} photosynthetic photon flux dencity (PPFD)

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(Hinklenton and Joliffe 1980, Peet et al. 1986, Kelly et al. 1991, Morin et al. 1992). This result was unexpected because the photosynthetic enhancement that occurs upon exposure to elevated CO_2 is greater at high than at low PPFD. According to current photosynthesis models (Farquhar and von Caemmerer 1982), rates of ATP and NADPH synthesis are decreased at low PPFD and photosynthesis becomes limited by the rate of ribulose 1,5-bisphosphate (Ru1,5bisP) regeneration. The response of leaf carboxylation rates to CO_2 enrichment is diminished when Ru1,5bisP regeneration is the primary determinant of photosynthetic rate (Quick et al. 1991, Stitt et al. 1991). Feedback inhibition of photosynthesis is an additional factor affecting the response of plants to elevated CO_2 (Neales and Incoll 1968). In theory, suppressed rates of photosynthesis in elevated CO_2 are less likely to occur at low than at high PPFD, because feedback inhibition is not readily induced by light limiting conditions (Sharkey 1985).

The objectives of this research were to identify physiological responses of tobacco plants during growth in elevated CO_2 at low PPFD. Effects of elevated CO_2 on Rubisco, chlorophyll (Chl), protein and leaf carbohydrate levels were determined and results were compared with earlier CO_2 enrichment studies (Sage et al. 1989, Sicher et al. 1994).

Abbreviations – DAP, days after planting; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; Ru1,5bisP, ribulose 1,5-bisphosphate; SLW, specific leaf weight.

Materials and methods

Plant materials

Nicotiana tabacum (L.), either genotype W38 or Samsun, was grown in controlled environment chambers (model M-11, Environmental Growth Chambers, Chagrin Falls, OH, USA) at 25 to 27°C, essentially as described earlier (Sicher et al. 1994). The W38 germplasm was obtained from a segregating population of a transformant that contained antisense DNA sequences to the small subunit of Rubisco (line 3, Rodermel et al. 1988). However, all plants used in the current study were preselected for normal Rubisco levels. Seeds were distributed on the surface of 1.8-l pots filled with equal parts vermiculite and Jiffy-mix (Jiffy Products Inc, Batavia, IL, USA) and the pots were covered with clear plastic wrap to retain moisture. Seeds were allowed to germinate for 1 week at 27°C using 200 μmol m^{-2} s^{-1} PPFD. The plastic wrap was removed and seedlings were grown for an additional 2 weeks at 27°C on a 14 h day/10 h night cycle using 450 μmol m^{-2} s^{-1} PPFD and ambient relative humidity. Additional CO_2 (Potomac Air Gas Inc., Linthicum, MD, USA) was supplied as necessary to provide a chamber air CO_2 concentration of 35 ± 1 Pa. Approximately 21 days after planting (DAP), individual seedlings were transplanted to 3-l plastic pots to reduce root restriction. Elevated CO_2 treatments (70 ± 2 Pa) were initiated when plants were 5 weeks old by transferring one half of the plants to a matching controlled environment chamber. Plants were harvested 9 or 10 days later for leaf area and dry weight determinations.

Photosynthesis measurements

Net photosynthesis rates were measured on the sixth (W38) or seventh (Samsun) leaf from emergence using a portable, closed Photosynthesis system (model 6200, Li-Cor Inc., Lincoln, NE, USA) equipped with a 1-l clamp-on leaf cuvette that exposed 10 cm^2 of leaf area. Light, temperature, humidity and CO_2 levels were as for plant growth, unless indicated otherwise. Photosynthesis rates also were measured at 900 μmol m^{-2} s^{-1} PPFD and 70 Pa CO_2 following a 1 h acclimation period. After photosynthesis rate was determined, four to six leaf discs (0.79 cm^2 each) were removed from the distal end of the leaf and these were rapidly frozen in liquid N_2 (Quick et al. 1991). Samples were stored at −80°C until analysis.

Biochemical procedures

Initial and total Rubisco activities were determined at 30°C using radiochemical assays essentially as described elsewhere (Quick et al. 1991, Sicher et al. 1994). Rubisco protein levels were measured using PAGE and a dye-binding method according to Makino et al. (1986). Standard curves were prepared from gel slices using purified tobacco Rubisco that was isolated and crystallized according to Servaites (1985). Soluble protein levels of each leaf extract were measured with Coomassie Brilliant Blue R using γ-globulin as the standard (Bradford 1976). Starch and sucrose were extracted and measured in coupled enzyme assays as described previously (Sicher et al. 1986). One leaf disc from each set of samples was used to measure Chl (Vernon 1960). Experiments reported here have been repeated at least once.

Results

Dry matter distribution

Total above ground dry matter and leaf area were determined 9 to 10 days after the elevated CO_2 treatment was initiated. Total above ground plant dry weights of genotypes W38 and Samsun were 36 and 28% greater ($P < 0.05$), respectively, in CO_2-enriched than in ambient-CO_2 air (Tab. 1). Over one-half of the added biomass was allocated to leaves of both genotypes ($P < 0.05$). Leaf area of W38 was unchanged ($P > 0.05$) and that of Samsun was less ($P < 0.05$) at 70 than at 35 Pa CO_2. As observed with bean plants grown at low PPFD (Porter and Grodzinski 1984, Hoddinott and Jolliffe 1988), specific leaf weight (SLW) of both tobacco genotypes was greater ($P < 0.05$) in elevated than in ambient CO_2.

Tab. 1. Dry matter partitioning of two tobacco genotypes in response to doubling the ambient CO_2 concentration.

Genotype	n	CO_2 (Pa)	Total DW (g)	Leaf DW (g)	Leaf area (m^2)	SLW ($g\ m^{-2}$)
W38	12	35	24.9 ± 1.1	13.7 ± 0.4	0.38 ± 0.03	37.3 ± 1.9
	7	70	33.8 ± 1.4	18.3 ± 1.1	0.38 ± 0.03	50.1 ± 5.5
Samsun	4	35	25.1 ± 0.5	19.5 ± 0.4	0.69 ± 0.01	28.2 ± 0.3
	4	70	32.2 ± 1.5	24.4 ± 1.2	0.60 ± 0.04	41.2 ± 0.7

Net photosynthesis rates

Net photosynthesis rates of W38 plants during 9 or 10 days of growth in either 35 or 70 Pa CO_2 air are shown in Fig. 1. The photosynthesis measurements in Fig. 1A were performed at the respective treatment CO_2 concentrations using a limiting irradiance of 450 μmol m^{-2} s^{-1} PPFD (Farquhar and von Caemmerer 1982, Stitt et al. 1991). Photosynthesis rates were 9% greater ($P < 0.05$) on average in elevated CO_2 than in ambient CO_2. Mean photosynthesis rates, averaged over the 9 day treatment period, were 13.0 ± 0.4 and 14.2 ± 0.5 μmol m^{-2} s^{-1}, when plants were grown and measured in either 35 or 70 Pa CO_2 air, respectively. When results from the first day's measurements were omitted, net photosynthesis rates of ambient-CO_2-grown and elevated-CO_2-grown plants changed less than 2 μmol m^{-2} s^{-1} between days 1 and 9 of the study.

When measured at 900 μmol m^{-2} s^{-1} PPFD and 70 Pa CO_2, net photosynthesis rates of ambient-CO_2-grown W38 were 27.2 ± 0.7 and 25.4 ± 2.1 μmol m^{-2} s^{-1} on the first and last sampling dates, respectively (Fig. 1B). In contrast, photosynthesis rates of plants grown in CO_2 enriched air decreased from about 30 to 15 μmol m^{-2} s^{-1} between the beginning and the end of the study. Net assimilation rates of ambient-CO_2-grown and elevated-CO_2-grown plants were significantly different ($P < 0.05$) 7 to 10 days after the elevated CO_2 treatment was initiated.

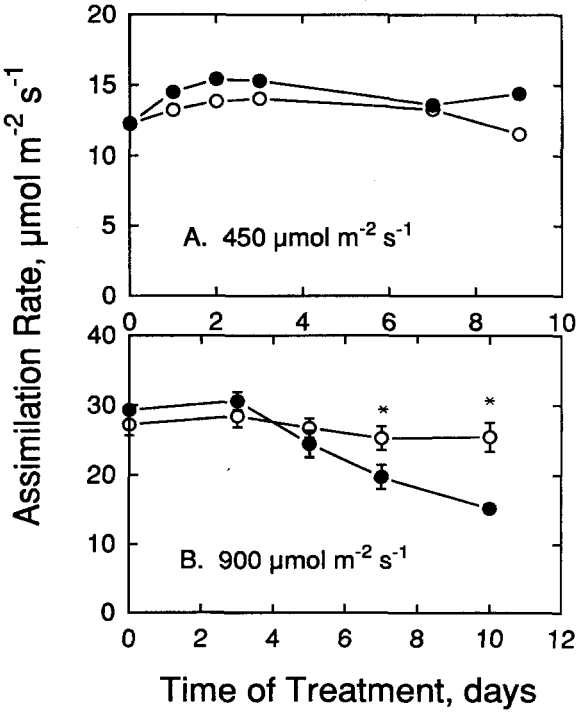

Fig. 1. Effects of CO_2 enrichment on photosynthetic rates of genotype W38 tobacco plants grown at 450 μmol m^{-2} s^{-1} PPFD. A. Net CO_2 assimilation rates were measured using environmental conditions employed for plant growth at either 35 Pa (○) or 70 Pa (●) CO_2 air. B. Conditions and symbols were as above except that assimilation rates were measured at 70 Pa CO_2 and 900 μmol m^{-2} s^{-1} PPFD. Values are means ± SE for n = 4 to 8. *, Significant difference ($P < 0.05$).

Chl and protein

Changes in Chl and soluble leaf protein of W38 in response to 70 Pa CO_2 air are shown in Fig. 2A,B, respectively. Leaves of both ambient-CO_2-grown and elevated-CO_2-grown plants contained less than 0.4 g m^{-2} Chl at the

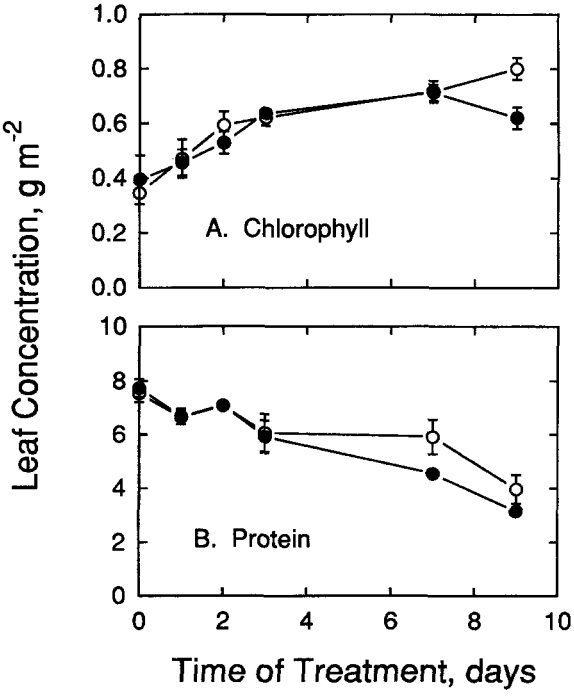

Fig. 2. Effects of CO_2 enrichment on Chl (A) and leaf soluble protein content (B). Experimental details and symbols were as in Fig. 1 except that 450 μmol m^{-2} s^{-1} PPFD was used throughout.

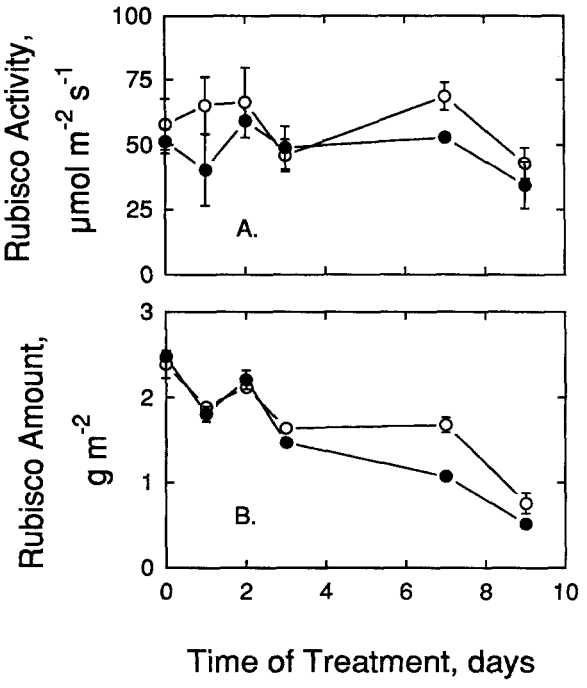

Fig. 3. Response of Rubisco activity (A) and leaf Rubisco protein (B) to CO_2 enrichment. Experimental details and symbols were as in Fig. 1 except that 450 μmol m^{-2} s^{-1} PPFD was used throughout.

start of the experiment and 9 days later these values increased to over 0.6 g m^{-2}. Soluble leaf protein levels of ambient-CO_2-grown and elevated-CO_2-grown plants decreased an average of 53% between the first and last sampling. When averaged over all sampling dates, neither Chl nor soluble leaf protein content were significantly different ($P > 0.05$) with respect to CO_2 enrichment at low PPFD.

Rubisco activity and concentration

Rubisco activity of each sample was measured at 30°C, both before and after in vitro activation with CO_2 and Mg^{2+}. In agreement with earlier findings (Delgado et al. 1993, Sicher et al. 1994), these two measurements were not significantly different ($P > 0.05$) in tobacco leaf extracts. Moreover, Rubisco was fully activated throughout the study in both CO_2 treatments (data not shown). Rubisco activities of ambient-CO_2-grown and elevated-CO_2-grown plants were 59 ± 4.5 and 49 ± 3.7 μmol m^{-2} s^{-1} respectively ($P < 0.05$), when averaged over the entire 9 day study (Fig. 3A). Carboxylase rates in both the 35 and 70 Pa CO_2 environments decreased by about 30% between days 0 and 9.

Leaf Rubisco protein levels, averaged over the two CO_2 treatments, decreased 74% between the beginning and the end of the study (Fig. 3B). In agreement with soluble protein measurements, Rubisco concentrations during the 9 day study were not significantly different ($P > 0.05$) between CO_2 treatments.

Leaf carbohydrates

One frequently reported response of plants to elevated CO_2 partial pressures is increased leaf reserve formation (Finn and Brun 1982, Peet et al. 1986, Yelle et al. 1989a). Mean diurnal fluctuations in leaf starch of ambient-CO_2-grown plants were 4.8 mmol hexose equivalents m^{-2} when averaged over all 7 harvest dates (Fig. 4A). This value increased to 11.0 mmol hexose equivalents m^{-2} when the chamber air CO_2 partial pressure was doubled from 35 to 70 Pa ($P < 0.05$). Leaf starch levels determined during the first hour of the photoperiod changed by an average daily increment of −0.4 and 3.7 mmol hexose equivalents m^{-2} in the 35 and 70 Pa CO_2 environments, respectively ($P < 0.05$).

Unlike starch, leaf sucrose concentrations during the first and last hour of the photoperiod were not significantly different ($P > 0.05$) in either the ambient or elevated CO_2 environments (Fig. 4B). However, sucrose levels were about 85% higher on average in 70 than in 35 Pa CO_2 air ($P < 0.05$).

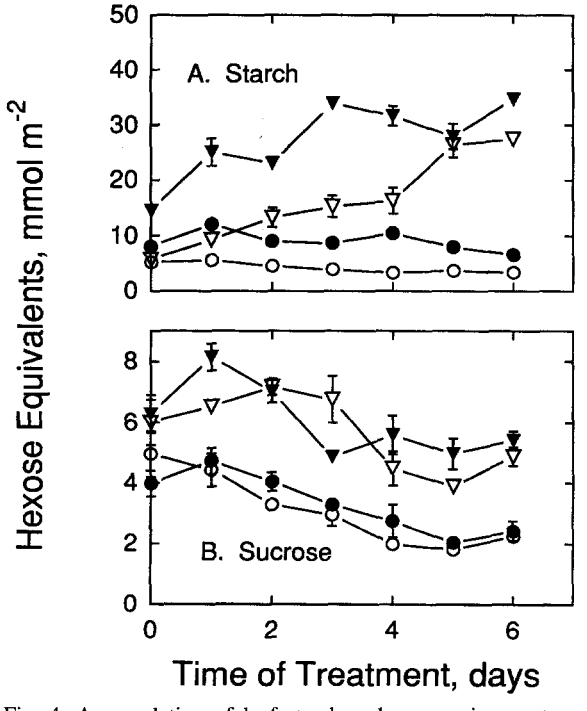

Fig. 4. Accumulation of leaf starch and sucrose in genotype Samsun tobacco plants in response to CO_2 enrichment. Leaf starch (A) and sucrose (B) were determined using leaf discs sampled at 1 h (○, ●) and 13 h (△, ▲) of the photoperiod. Plants were grown with 450 μmol m^{-2} s^{-1} PPFD for 1 week in either ambient (○, △) or elevated CO_2 (●, ▲) air.

Discussion

Down regulation of photosynthesis was observed in previous studies employing *N. tabacum* after 1 to 3 weeks of elevated-CO_2 treatment (Raper and Peedin 1978, Sicher et al. 1994). In the current study, doubling the ambient CO_2 concentration for 9 days resulted in a 9% increase in net photosynthesis rates when measurements were performed using the same environmental conditions as those used for plant growth. This finding suggested that photosynthesis was not down regulated by growth in elevated CO_2 chamber air at low PPFD. A small, CO_2-dependent stimulation of photosynthesis was expected because light-limiting conditions were used for both growth and photosynthesis rate measurements (Farquhar and von Caemmerer 1982, Sharkey 1985). The apparent discrepancy between earlier observations and the current study was resolved when 900 μmol m^{-2} s^{-1} PPFD and 70 Pa CO_2 were employed for measuring net CO_2 exchange. Down regulation of photosynthesis during growth in elevated CO_2, both here and earlier (Sicher et al. 1994), was observed 4 to 7 days after CO_2 enrichment was initiated. These results demonstrate the importance of employing more than one set of environmental conditions for measuring CO_2 fixation rates during photosynthetic acclimation experiments and show that photosynthetic acclimation occurred in tobacco grown at low PPFD.

In spite of the small response of photosynthesis rate to CO_2 enrichment at low PPFD, changes in plant growth and leaf metabolism were observed both in this study and in prior controlled environment studies (Peet et al. 1986, Hoddinott and Jolliffe 1988, Morin et al. 1992). Both leaf and stem dry weight gain were greater in 70 than in 35 Pa CO_2 air. However, the total plant dry weight gain due to CO_2 enrichment was less than that observed previously when plants were grown with 900 μmol m^{-2} s^{-1} PPFD (Sicher et al. 1994). As in earlier studies performed at low PPFD (Porter and Grodzinski 1984, Hoddinott and Jolliffe 1988), the SLW of tobacco was greater in elevated than in ambient CO_2. This was accomplished without an increase in leaf area and was probably related to an accumulation of starch and sucrose in response to CO_2 enrichment. Morin et al. (1992) reported an accumulation of leaf starch at low PPFD in clover (*Trifolium subterraneum* L.) grown in elevated compared to ambient CO_2. These authors also showed that leaf starch levels were proportional to the duration of CO_2 enrichment.

In comparison to results at a moderate PPFD (Sicher et al. 1994), soluble leaf protein and Rubisco protein decreased and Chl increased when a PPFD of 450 μmol m^{-2} s^{-1} was used. These changes in protein and Chl content typically are observed in plants during acclimation to low PPFD (Björkman 1968). Differences in the concentration of soluble protein, Rubisco content and leaf Chl levels between ambient and CO_2 enriched plants grown at moderate PPFD (Sicher et al. 1994) were not evident in the current study. Clearly, the impact of CO_2 enrichment on leaf development was diminished at low compared to moderate PPFD.

Decreases in Rubisco activity have been observed in many C_3 species during acclimation to elevated CO_2 environments (Wong 1979, Sage et al. 1989, Rowland-Bamford et al. 1990). Growth at 70 Pa CO_2 resulted in decreased Rubisco activity, both in the current study and earlier (Sicher et al. 1994). Sage et al. (1989) reported that Rubisco activation state declined in response to CO_2 enrichment in all five species they examined. Evidence for deactivation of Rubisco in *N. tabacum* leaves during growth in elevated CO_2 is currently lacking. Changes in Rubisco activity in response to elevated CO_2 also were more difficult to detect at low than at moderate PPFD (Sicher et al. 1994). Overall, Rubisco activity of plants raised with a PPFD of 450 μmol m^{-2} s^{-1} was less than half that of plants grown under moderate irradiance. However, photosynthetic studies of transgenic plants with decreased Rubisco levels demonstrated that Rubisco-imposed limitations on photosynthetic rates decreased as the CO_2 concentration increased (Quick et al. 1991).

Light is a limiting factor in many natural ecosystems and its effects on the down regulation of photosynthesis are poorly understood (Bunce 1992). The impact of CO_2 enrichment on the growth of *N. tabacum* in the current controlled environment study was diminished by lowering the irradiance from 900 to 450 μmol m^{-2} s^{-1} PPFD. Nevertheless, total plant dry matter, SLW, net CO_2 assimilation rate, leaf carbohydrate level and extractable Rubisco activity were affected by CO_2 enrichment both in this and in an earlier study performed at moderate PPFD (Sicher et al. 1994). Also, photosynthetic acclimation was observed in *N. tabacum* using low light growth conditions provided that CO_2 assimilation rate measurements were performed with high irradiance and elevated CO_2.

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